

10/587523

AP20 Rec'd PCT/PTO 27 JUL 2006

<110> AXXAM SRL

<120> photoproteins with enhanced bioluminescence and assays using the same

<130> 1009PCT

<160> 22

<170> PatentIn version 3.1

<210> 1

<211> 198

<212> PRT

<213> Clytia gregaria

<400> 1

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Asp Asn Pro Lys Trp Val Asn Arg His Lys Phe Met Phe Asn Phe Leu
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Asp Ile Asn Gly Asp Gly Lys Ile Thr Leu Asp Glu Ile Val Ser Lys
35 40 45

Ala Ser Asp Asp Ile Cys Ala Lys Leu Gly Ala Thr Pro Glu Gln Thr
50 55 60

Lys Arg His Gln Asp Ala Val Glu Ala Phe Phe Lys Lys Ile Gly Met
65 70 75 80

Asp Tyr Gly Lys Glu Val Glu Phe Pro Ala Phe Val Asp Gly Trp Lys
85 90 95

Glu Leu Ala Asn Tyr Asp Leu Lys Leu Trp Ser Gln Asn Lys Lys Ser
100 105 110

2

Leu Ile Arg Asp Trp Gly Glu Ala Val Phe Asp Ile Phe Asp Lys Asp
115 120 125

Gly Ser Gly Ser Ile Ser Leu Asp Glu Trp Lys Ala Tyr Gly Arg Ile
130 135 140

Ser Gly Ile Cys Ser Ser Asp Glu Asp Ala Glu Lys Thr Phe Lys His
145 150 155 160

Cys Asp Leu Asp Asn Ser Gly Lys Leu Asp Val Asp Glu Met Thr Arg
165 170 175

Gln His Leu Gly Phe Trp Tyr Thr Leu Asp Pro Asn Ala Asp Gly Leu
180 185 190

Tyr Gly Asn Phe Val Pro
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<212> PRT

<213> Unknown

<220>

<223> Clytin mutant: mutClyK1

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20 25 30

Asp Ile Asn Gly Asp Gly Lys Ile Thr Leu Asp Glu Ile Val Ser Lys
35 40 45

Ala Ser Asp Asp Ile Ser Ala Lys Leu Gly Ala Thr Pro Glu Gln Thr
50 55 60

Lys Arg His Gln Asp Ala Val Glu Ala Phe Phe Lys Lys Ile Gly Met
65 70 75 80

Asp Tyr Gly Lys Glu Val Glu Phe Pro Ala Phe Val Asp Gly Trp Lys
85 90 95

3

Glu Leu Ala Asn Tyr Asp Leu Lys Leu Trp Ser Gln Asn Lys Lys Ser
 100 105 110

Leu Ile Arg Asp Trp Gly Glu Ala Val Phe Asp Ile Phe Asp Lys Asp
 115 120 125

Gly Ser Gly Ser Ile Ser Leu Asp Glu Trp Lys Ala Tyr Gly Arg Ile
 130 135 140

Ser Gly Ile Cys Ser Ser Asp Glu Asp Ala Glu Lys Thr Phe Lys His
 145 150 155 160

Cys Asp Leu Asp Asn Ser Gly Lys Leu Asp Val Asp Glu Met Thr Arg
 165 170 175

Gln His Leu Gly Phe Trp Tyr Thr Leu Asp Pro Asn Ala Asp Gly Leu
 180 185 190

Tyr Gly Asn Phe Val Pro
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<210> 3

<211> 198

<212> PRT

<213> Unknown

<220>

<223> Clytin mutant: mutClyK4

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 20 25 30

Asp Ile Asn Gly Asp Gly Lys Ile Thr Leu Asp Glu Ile Val Ser Lys
 35 40 45

Ala Ser Asp Asp Ile Cys Ala Lys Leu Gly Ala Thr Pro Glu Gln Thr
 50 55 60

Lys Arg His Gln Asp Ala Val Glu Ala Phe Phe Lys Lys Ile Gly Met
 65 70 75 80

4

Asp Tyr Gly Lys Glu Val Glu Phe Pro Ala Phe Val Asp Gly Trp Lys
85 90 95

Glu Leu Ala Asn Tyr Asp Leu Lys Leu Trp Ser Gln Asn Lys Lys Ser
100 105 110

Leu Ile Arg Asp Trp Gly Glu Ala Val Phe Asp Ile Phe Asp Lys Asp
115 120 125

Gly Ser Gly Cys Ile Ser Leu Asp Glu Trp Lys Ala Tyr Gly Arg Ile
130 135 140

Ser Gly Ile Cys Ser Ser Asp Glu Asp Ala Glu Lys Thr Phe Lys His
145 150 155 160

Cys Asp Leu Asp Asn Ser Gly Lys Leu Asp Val Asp Glu Met Thr Arg
165 170 175

Gln His Leu Gly Phe Trp Tyr Thr Leu Asp Pro Asn Ala Asp Gly Leu
180 185 190

Tyr Gly Asn Phe Val Pro
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<211> 198

<212> PRT

<213> Unknown

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<223> Clytin mutant: 1F10 mutant

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Asp Asn Pro Lys Trp Val Asn Arg His Lys Phe Met Phe Asn Phe Leu
20 25 30

Asp Ile Asn Gly Asp Gly Lys Ile Thr Leu Asp Glu Ile Val Ser Arg
35 40 45

Ala Ser Asp Asp Ile Cys Ala Lys Leu Gly Ala Thr Pro Glu Gln Thr
50 55 60

5

Lys Arg His Gln Asp Ala Val Glu Ala Phe Phe Lys Lys Ile Gly Met
65 70 75 80

Asp Tyr Gly Lys Glu Val Glu Phe Pro Ala Phe Val Asp Gly Trp Lys
85 90 95

Glu Leu Ala Asn Tyr Asp Leu Lys Leu Trp Ser Gln Asn Lys Lys Ser
100 105 110

Leu Ile Arg Asp Trp Gly Glu Ala Val Phe Asp Ile Phe Asp Lys Asp
115 120 125

Gly Ser Gly Ser Ile Ser Leu Asp Glu Trp Lys Ala Tyr Gly Arg Ile
130 135 140

Ser Gly Ile Cys Ser Ser Asp Glu Asp Ala Glu Lys Thr Phe Lys His
145 150 155 160

Cys Asp Leu Asp Asn Ser Gly Lys Leu Asp Val Asp Glu Met Thr Arg
165 170 175

Gln His Leu Gly Phe Trp Tyr Thr Leu Asp Pro Asn Ala Asp Gly Leu
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Tyr Gly Asp Phe Val Pro
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<211> 198

<212> PRT

<213> Unknown

<220>

<223> Clytin mutant: 1H7 mutant

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Asp Asn Pro Lys Trp Val Asn Arg His Lys Phe Met Phe Asn Phe Leu
20 25 30

Asp Ile Asn Gly Asp Gly Lys Ile Thr Leu Asp Glu Ile Val Ser Lys
35 40 45

6

Ala Ser Asp Asp Ile Cys Ala Lys Leu Gly Ala Thr Pro Glu Gln Thr
 50 55 60

Lys Arg His Arg Asp Ala Val Glu Ala Phe Phe Lys Lys Ile Gly Met
 65 70 75 80

Asp Tyr Gly Lys Glu Val Glu Phe Pro Val Phe Val Asp Gly Trp Lys
 85 90 95

Glu Leu Ala Asn Tyr Asp Leu Lys Leu Trp Ser Gln Asn Lys Lys Ser
 100 105 110

Leu Ile Arg Asp Trp Gly Glu Ala Val Phe Asp Ile Phe Asp Lys Asp
 115 120 125

Gly Ser Gly Ser Ile Ser Leu Asp Glu Trp Lys Ala Tyr Gly Arg Ile
 130 135 140

Ser Gly Ile Cys Ser Ser Asp Glu Asp Ala Glu Lys Thr Phe Lys His
 145 150 155 160

Cys Asp Leu Asp Asn Ser Gly Lys Leu Asp Val Asp Glu Met Thr Arg
 165 170 175

Gln His Leu Gly Phe Trp Tyr Ile Leu Asp Pro Asn Ala Asp Gly Leu
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Tyr Gly Asn Phe Val Pro
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<210> 6

<211> 198

<212> PRT

<213> Unknown

<220>

<223> Clytin mutant: 1C12 mutant

<400> 6

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 20 25 30

7

Asp Ile Asn Gly Asp Gly Lys Ile Thr Leu Asp Glu Ile Val Ser Lys
35 40 45

Ala Ser Asp Asp Ile Cys Ala Lys Leu Gly Ala Thr Pro Glu Gln Thr
50 55 60

Lys Arg His Gln Asp Ala Val Glu Ala Phe Phe Lys Lys Ile Gly Met
65 70 75 80

Asp Phe Gly Lys Glu Val Glu Phe Pro Ala Phe Val Asp Gly Trp Lys
85 90 95

Glu Leu Ala Asn Tyr Asp Leu Lys Leu Trp Ser Gln Asn Asn Lys Ser
100 105 110

Leu Ile Arg Asp Trp Gly Glu Ala Val Phe Asp Ile Leu Asp Lys Asp
115 120 125

Gly Ser Gly Ser Ile Ser Leu Asp Glu Trp Lys Ala Tyr Gly Arg Ile
130 135 140

Ser Gly Ile Cys Arg Ser Asp Glu Asp Ala Glu Lys Thr Phe Lys His
145 150 155 160

Cys Asp Leu Asp Asn Ser Gly Lys Leu Asp Val Asp Glu Met Thr Arg
165 170 175

Gln His Leu Gly Phe Trp Tyr Thr Leu Asp Pro Asn Ala Asp Gly Leu
180 185 190

Tyr Gly Asn Phe Val Pro
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<210> 7

<211> 198

<212> PRT

<213> Unknown

<220>

<223> Clytin mutant: 25N03b mutant

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Met Ala Asp Thr Ala Ser Lys Tyr Ala Val Lys Leu Arg Pro Asn Phe
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8

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Asp Ile Asn Gly Asp Gly Lys Ile Thr Leu Asp Glu Ile Val Ser Lys
 35 40 45

Ala Ser Asp Asp Ile Cys Ala Lys Leu Gly Ala Thr Pro Glu Gln Thr
 50 55 60

Lys Arg His Gln Asp Ala Val Glu Ala Phe Phe Lys Lys Ile Gly Met
 65 70 75 80

Asp Tyr Gly Lys Glu Val Glu Phe Pro Ala Phe Val Asp Gly Trp Lys
 85 90 95

Glu Leu Ala Asn Tyr Asp Leu Lys Leu Trp Ser Gln Asn Lys Lys Ser
 100 105 110

Leu Ile Arg Asp Trp Gly Glu Ala Val Phe Asp Ile Phe Asp Lys Asp
 115 120 125

Gly Ser Gly Ser Ile Ser Leu Asp Glu Trp Lys Ala Tyr Cys Arg Ile
 130 135 140

Ser Gly Ile Cys Ser Ser Asp Glu Asp Ala Glu Lys Thr Phe Lys His
 145 150 155 160

Cys Asp Leu Asp Asn Ser Gly Lys Leu Asp Val Asp Glu Met Thr Arg
 165 170 175

Gln His Leu Gly Phe Trp Tyr Thr Leu Asp Pro Asn Ala Asp Gly Leu
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Tyr Gly Asn Phe Val Pro
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<210> 8

<211> 198

<212> PRT

<213> Unknown

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<223> Clytin mutant: 3C12 mutant

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Met Ala Asp Thr Ala Ser Lys Tyr Ala Val Lys Leu Arg Pro Asn Phe
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 20 25 30

Asp Ile Asn Gly Asp Gly Lys Ile Thr Leu Asp Glu Ile Val Ser Lys
 35 40 45

Ala Ser Asp Asp Val Cys Ala Lys Leu Gly Ala Thr Pro Glu Gln Thr
 50 55 60

Lys Arg His Gln Asp Ala Val Glu Ala Phe Phe Lys Lys Ile Gly Met
 65 70 75 80

Asp Tyr Gly Lys Glu Val Glu Phe Pro Ala Phe Val Asp Gly Trp Lys
 85 90 95

Glu Leu Ala Asn Tyr Asp Leu Lys Leu Trp Ser Gln Asn Lys Lys Ser
 100 105 110

Leu Ile Arg Asp Trp Gly Glu Ala Val Phe Asp Ile Phe Asp Lys Asp
 115 120 125

Gly Ser Gly Ser Ile Ser Leu Asp Glu Trp Lys Ala Tyr Gly Arg Ile
 130 135 140

Ser Gly Ile Cys Arg Ser Asp Glu Asp Ala Glu Lys Thr Phe Lys His
 145 150 155 160

Cys Asp Leu Asp Asn Ser Gly Lys Leu Asp Val Asp Glu Met Thr Arg
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Gln His Leu Gly Phe Trp Tyr Thr Leu Asp Pro Asn Ala Asp Gly Leu
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Tyr Gly Asn Phe Val Pro
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<210> 9

<211> 198

<212> PRT

<213> Unknown

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<223> Clytin mutant: 6H22 mutant

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 20 25 30

Asp Ile Asn Gly Asp Gly Lys Val Thr Leu Asp Glu Ile Val Ser Lys
 35 40 45

Ala Ser Asp Asp Ile Cys Ala Arg Leu Gly Ala Thr Pro Glu Gln Thr
 50 55 60

Lys Arg His Gln Asp Ala Val Glu Ala Phe Phe Lys Lys Ile Gly Met
 65 70 75 80

Asp Tyr Gly Lys Glu Val Glu Phe Pro Ala Phe Val Asp Gly Trp Lys
 85 90 95

Glu Leu Ala Asn Tyr Asp Leu Lys Leu Trp Ser Gln Asn Lys Lys Ser
 100 105 110

Leu Ile Arg Asp Trp Gly Glu Ala Val Phe Asp Ile Phe Asp Lys Asp
 115 120 125

Gly Ser Gly Ser Ile Ser Leu Asp Glu Trp Lys Ala Tyr Gly Arg Ile
 130 135 140

Ser Gly Ile Cys Ser Ser Asp Glu Asp Ala Glu Lys Thr Phe Lys His
 145 150 155 160

Cys Asp Leu Asp Asn Ser Gly Lys Leu Asp Val Asp Glu Met Thr Arg
 165 170 175

Gln His Leu Gly Phe Trp Tyr Thr Leu Asp Pro Asn Ala Asp Gly Leu
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Tyr Gly Asn Phe Val Pro
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<211> 198

<212> PRT

<213> Unknown

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<223> Clytin mutant: 12mutCly

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			20					25						30	

Asp	Ile	Asn	Gly	Asp	Gly	Lys	Ile	Thr	Leu	Asp	Glu	Ile	Val	Ser	Lys
		35					40						45		

Ala	Ser	Asp	Asp	Ile	Cys	Ala	Lys	Leu	Glu	Ala	Thr	Pro	Glu	Gln	Thr
	50					55					60				

Lys	Arg	His	Gln	Val	Cys	Val	Glu	Ala	Phe	Phe	Arg	Gly	Cys	Gly	Met
65					70					75					80

Glu	Tyr	Gly	Lys	Glu	Ile	Ala	Phe	Pro	Gln	Phe	Leu	Asp	Gly	Trp	Lys
				85					90						95

Gln	Leu	Ala	Thr	Ser	Glu	Leu	Lys	Lys	Trp	Ala	Arg	Asn	Glu	Pro	Thr
						100			105					110	

Leu	Ile	Arg	Glu	Trp	Gly	Asp	Ala	Val	Phe	Asp	Ile	Phe	Asp	Lys	Asp
			115					120						125	

Gly	Ser	Gly	Ser	Ile	Ser	Leu	Asp	Glu	Trp	Lys	Ala	Tyr	Gly	Arg	Ile
						130					135			140	

Ser	Gly	Ile	Cys	Ser	Ser	Asp	Glu	Asp	Ala	Glu	Lys	Thr	Phe	Lys	His
145						150				155					160

Cys	Asp	Leu	Asp	Asn	Ser	Gly	Lys	Leu	Asp	Val	Asp	Glu	Met	Thr	Arg
						165				170					175

Gln	His	Leu	Gly	Phe	Trp	Tyr	Thr	Leu	Asp	Pro	Asn	Ala	Asp	Gly	Leu
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Tyr	Gly	Asn	Phe	Val	Pro
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<211> 600

<212> DNA

<213> Unknown

<220>

<223> Clytin mutant: mutClyK1_dna

<400> 11

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accctggacg agatcgtgag caaggccagc gacgacatct gcgccaagct gggcgccacc    180
cccgagcaga ccaagagaca ccaggacgcc gtggaggcct tcttcaagaa gatcggcatg    240
gactacggca aggaggtgga gttccccgcc ttcgtggacg gctggaagga gctggccaac    300
taccacctga agctgtggag ccagaacaag aagagcctca tcagggactg gggcgaggcc    360
gtgttcgaca tcttcgacaa ggacggcagc ggctgcatca gcctggatga gtggaaggcc    420
tacggcagaa tcagcggcat ctgcagcagc gacgaggacg ccgaaaagac cttcaagcac    480
tgcgacctgg acaacagcgg caagctggac gtggacgaga tgaccagaca gcacctggac    540
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<210> 12

<211> 600

<212> DNA

<213> Unknown

<220>

<223> Clytin mutant: mutClyK4_dna

<400> 12

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accctggacg agatcgtgag caaggccagc gacgacatct gcgccaagct gggcgccacc    180
cccgagcaga ccaagagaca ccaggacgcc gtggaggcct tcttcaagaa gatcggcatg    240
gactacggca aggaggtgga gttccccgcc ttcgtggacg gctggaagga gctggccaac    300
tacgacctga agctgtggag ccagaacaag aagagcctca tcagggactg gggcgaggcc    360

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13

gtgttcgaca tcttcgacaa ggacggcagc ggctgcatca gcctggatga gtggaaggcc 420
 tacggcagaa tcagcggcat ctgcagcagc gacgaggacg ccgaaaagac cttcaagcac 480
 tgcgacctgg acaacagcgg caagctggac gtggacgaga tgaccagaca gcacctgggc 540
 ttctggtaca ccctggaccc caatgccgac ggctgtacg gcaacttcgt gccttgataa 600

<210> 13

<211> 600

<212> DNA

<213> Unknown

<220>

<223> Clytin mutant: 1F10 mutant_dna

<400> 13

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 accctggacg agatcgtgag cagggccagc gacgacatct gcgccaagct gggcgccacc 180
 cccgagcaga ccaagagaca ccaggacgcc gtggaggcct tcttcaagaa gatcggcatg 240
 gactacggca aggaggtgga gttccccgcc ttcgtggacg gctggaagga gctggccaac 300
 tacgacctga agctgtggag ccagaacaag aagagcctca tcagggactg gggcgaggcc 360
 gtgttcgaca tcttcgacaa ggacggcagc ggcagcatca gcctggatga gtggaaggcc 420
 tacggcagaa tcagcggcat ctgcagcagc gacgaggacg ccgaaaagac cttcaagcac 480
 tgcgacctgg acaacagcgg caagctggac gtggacgaga tgaccagaca gcacctgggc 540
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<210> 14

<211> 600

<212> DNA

<213> Unknown

<220>

<223> Clytin mutant: 1H7 mutant_dna

<400> 14

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accctggacg agatcgtgag caaggccagc gacgacatct gcgccaagct gggcgccacc	180
cccgagcaga ccaagagaca ccgggacgcc gtggaggcct tcttcaagaa gatcggcatg	240
gactacggca aggaggtgga gttccccgtc ttcgtggacg gctggaagga gctggccaac	300
tacgacctga agctgtggag ccagaacaag aagagcctca tcagggactg gggcgaggcc	360
gtgtttgaca tcttcgacaa ggacggcagc ggcagcatta gcctggatga gtggaaggcc	420
tacggtagaa tcagcggcat ctgcagcagc gacgaggacg ccgaaaagac cttcaagcac	480
tgcgacctgg acaacagcgg caagctggac gtggacgaga tgaccagaca gcacctgggc	540
ttctggtaca tcctggaccc caacgccgac ggcctgtacg gcaacttcgt gccttgataa	600

<210> 15

<211> 600

<212> DNA

<213> Unknown

<220>

<223> Clytin mutant: 1C12 mutant_dna

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accctggacg agatcgtgag caaggccagc gacgacatct gcgccaagct gggcgccacc	180
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gacttcggca aggaggtgga gttccccgcc ttcgtggacg gctggaagga gctggccaac	300
tacgacctga agctgtggag ccagaacaat aagagcctca tcagggactg gggcgaggcc	360
gtgttcgaca tcctcgacaa ggacggcagc ggcagcatca gcctggatga gtggaaggcc	420
tacggcagaa tcagcggcat ctgcagaagc gacgaggacg ccgaaaagac cttcaagcac	480
tgcgacctgg acaacagcgg caagctggac gtggacgaga tgaccagaca gcacctgggc	540
ttctggtaca ccctggaccc caacgccgac ggcctgtacg gcaacttcgt gccttgataa	600

<210> 16

<211> 600

<212> DNA

<213> Unknown

<220>

<223> Clytin mutant: 25N03b mutant_dna

<400> 16

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accctggacg agatcgtgag caaggccagc gacgacatct gcgccaagct gggcgccacc    180
cccgagcaga ccaagagaca ccaggacgcc gtggaggcct tcttcaagaa gatcggcatg    240
gactacggca aggaggtgga gttccccgcc ttcttgga gctggaagga gctggccaac    300
tacgacctga agctgtggag ccagaacaag aagagcctca tcagggactg gggcgaggcc    360
gtgttcgaca tcttcgacaa ggacggcagc ggcagcatca gcctggatga gtggaaggcc    420
tactgcagaa tcagcggcat ctgcagcagc gacgaggacg ccgaaaagac cttcaagcac    480
tgcgacctgg acaacagcgg caagctggac gtggacgaga tgaccagaca gcacctgggc    540
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<210> 17

<211> 600

<212> DNA

<213> Unknown

<220>

<223> Clytin mutant: 3C12 mutant_dna

<400> 17

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accctggacg agatcgtgag caaggccagc gacgacgtct gcgccaagct gggcgccacc    180
cccgagcaga ccaagagaca ccaggacgcc gtggaggcct tcttcaagaa gatcggcatg    240
gactacggca aggaggtgga gttccccgcc ttcttgga gctggaagga gctggccaac    300
tacgacctga agctgtggag ccaaaacaag aagagcctca tcagggactg gggcgaggcc    360
gtgttcgaca tcttcgacaa ggacggcagc ggcagcatca gcctggacga gtggaaggcc    420
tacggcagaa tcagcggcat ctgcagaagc gacgaggacg ccgaaaagac cttcaagcac    480

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tgcgacctgg acaacagcgg caagctggac gtggacgaga tgaccagaca gcacctgggc 540
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<210> 18

<211> 600

<212> DNA

<213> Unknown

<220>

<223> Clytin mutant: 6H22 mutant_dna

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 accctggacg agatcgtgag caaggccagc gacgacatct gcgccaggct gggcgccacc 180
 cccgagcaga ccaagagaca ccaggacgcc gtggaggcct tcttcaagaa gatcggcatg 240
 gactacggca aagaggtgga gttccccgcc ttcgtggacg gctggaagga gctggccaac 300
 tacgacctga agctgtggag ccagaacaag aagagcctca tcagggactg gggcgaggcc 360
 gtgttcgaca tcttcgacaa ggacggcagc ggcagcatca gcctggatga gtggaaggcc 420
 tacggcagaa tcagcggcat ctgcagcagc gacgaggacg ccgaaaagac cttcaagcac 480
 tgcgacctgg acaacagcgg caagctggac gtggacgaga tgaccagaca gcacctgggc 540
 ttctggtaca ccctggaccc caacgccgac ggctgtacg gcaacttcgt gccttgataa 600

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<211> 597

<212> DNA

<213> Unknown

<220>

<223> Clytin mutant: 12mutCly_dna

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accctggacg agatcgtgag caaggccagc gacgacatct gcgccaagct ggaggccacc 180
 cccgagcaga ccaagcggca ccaagtgtgc gtggaggcct tcttccgcgg ctgcggcatg 240
 gagtacggca aggagatcgc cttccccagc ttcctggacg gctggaagca gctggccaca 300
 agcgagctga agaagtgggc ccggaacgag cccaccctga tccgcgagtg gggcgacgcc 360
 gtgttcgaca tcttcgacaa ggacggcagc ggcagcatct ctctggacga gtggaaggcc 420
 tacggccgga tcagcggcat ctgcagcagc gacgaggacg ccgagaaaac cttcaagcac 480
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<210> 20

<211> 32

<212> DNA

<213> Unknown

<220>

<223> synthetic primer

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